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(19)

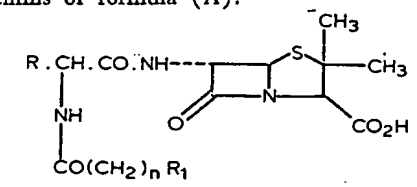
(54) 6-(α-HETEROCYCLYLCARBONYL AMINO-ACETAMIDO)-PENICILLINS AND COMPOSITIONS CONTAINING THEM

(71) We, BEECHAM GROUP LIMITED, a British Company, of Beecham House, Great West Road, Brentford, Middlesex, England, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to penicillin antibiotics and in particular to a class of α-(heterocyclic acylamino) penicillins which are of value as antibacterial agents.

Our British Patent Specification No. 1,130,445 discloses and claims α-(heterocyclic acylamino) penicillins of formula (A):

(A)

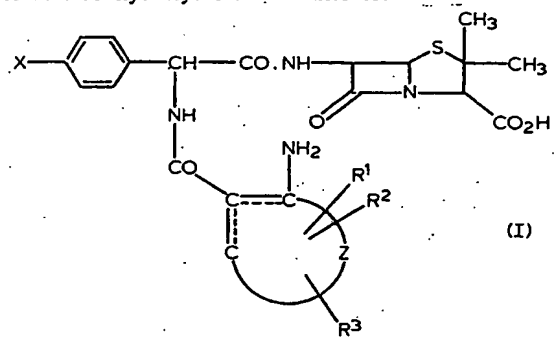


and non-toxic salts thereof, where R is a phenyl or thienyl group, R_1 is a heterocyclic group which may be substituted and n is zero or 1.

Within this class of penicillins, several sub-groups of penicillins have been described, for example those in British Patent Specifications Nos. 1,407,566 and 1,409,177 and U.S. Patent No. 3,864,329, which are characterised by having an oxygen function (such as a ketone, or an optionally etherified or esterified hydroxy group) on the heterocyclic ring.

It has now been found that α-(heterocyclic acylamino) penicillins having an amino substituent on the heterocyclic ring exhibit broad spectrum antibacterial activity and in particular are active against Pseudomonas organisms.

The present invention provides a penicillin of formula (I) or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof:



(I)

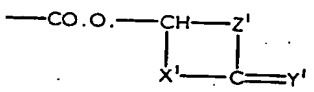
wherein X is hydrogen or hydroxy;

the dotted line represents a double bond in one of the positions shown;

Z represents the residue of a 6-membered heterocyclic ring containing one or two nitrogen atoms;

R^1, R^2 and R^3 are the same or different and each represents hydrogen, halogen, C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkylthio, cyano, amino, mercapto, C_{1-6}alkylamino, di-C_{1-6}alkylamino, C_{1-6}alkanoyl-amino, nitro, formyl or hydroxy or any two of R^1, R^2 and R^3 on adjacent carbon or nitrogen atoms represent the residue of a fused 5- or 6-membered carbocyclic or heterocyclic ring containing up to three heteroatoms selected from oxygen, sulphur and nitrogen, and being optionally substituted with up to three substituents selected from halogen, C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkylthio or hydroxy and the remaining symbol is as defined above.

The compounds of the present invention include pharmaceutically acceptable *in vivo* hydrolysable esters of compound (I). Suitable esters include those which hydrolyse readily in the human body to produce the parent acid, for example alkoxyalkyl esters such as methoxymethyl esters, acyloxyalkyl esters such as acetoxymethyl, pivaloyloxymethyl, α-acetoxyethyl, α-acetoxybenzyl and α-pivaloyloxyethyl esters; alkoxycarbonyloxyalkyl esters, such as ethoxycarbonyloxymethyl and α-ethoxycarbonyloxyethyl; and lactone, thiolactone and dithiolactone esters, i.e. ester groups of formula (II):



(II)

wherein X′ and Y′ are oxygen or sulphur and Z′ is an ethylene group or a 1,2-phenylene group optionally substituted by C_{1-6} alkoxy, halogen or nitro.

Preferred ester groups are the phthalidyl and 3,4-dimethoxyphthalidyl esters.

Suitable salts of the compound of formula (I) include metal salts, e.g. aluminium, alkali metal salts such as sodium or potassium, alkaline earth metal salts such as calcium or magnesium, and ammonium or substituted ammonium salts for example those with C_{1-6} alkylamino such as triethylamine, hydroxy-C_{1-6} alkylamines such as 2 - hydroxyethylamine, bis - (2 - hydroxyethyl) - amine, tris(hydroxymethyl)amine or tris - (2 - hydroxyethyl) - amine, cycloalkylamines such as bicyclohexylamine, or with procaine, dibenzylamine, N,N-dibenzylethylenediamine, 1-ephenamine, N-ethylpiperidine, N-benzyl-β-phenethylamine, dehydroabietylamine, N,N′-bis-dehydroabietylethylenediamine, or bases of the pyridine type such as pyridine, collidine or quinoline, or other amines which have been used to form salts with penicillins.

Pharmaceutically acceptable acid addition salts of such a compound are also included within this invention. Suitable acid addition salts of the compounds of formula (I) include, for example inorganic salts such as the sulphate, nitrate, phosphate, and borate; hydrohalides e.g. hydrochloride, hydrobromide and hydroiodide; and organic acid addition salts such as acetate, oxalate, tartrate, maleate, citrate, succinate, benzoate, ascorbate, methanesulphonate and *p*-toluenesulphonate, trifluoroacetate.

Suitable examples of the substituents R^1, R^2 and R^3 include chloro, bromo, fluoro, methyl, ethyl, *n* and *iso*-propyl, *n*-, *sec*- *iso*- and *tert*-butyl, methoxy, ethoxy, *n*- and *iso*-propoxy, *n*-, *sec*- *iso*- and *tert*-butoxy, methylthio, ethylthio, *n*- and *iso*-propylthio, cyano, amino, mercapto, nitro, methylamino, ethylamino, dimethylamino, diethylamino, acetylamino, formyl.

The moiety Z may complete a pyridine, pyrimidine, pyridazine, or 1,2,3-triazine ring.

When two of the groups R^1, R^2 and R^3 complete a further fused, saturated or unsaturated carboxylic or heterocyclic ring, examples of such rings include benzene, cyclohexane, cyclopentane, pyridine, pyrimidine, pyridazine, pyrazine; piperidine, piperazine, pyrrolidine, pyrazole, triazole, oxazole, triazine, thiazoline, thiazolidine, morpholine.

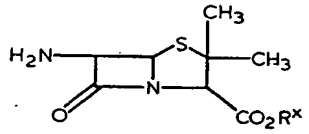
Such a fused ring may be attached to either a carbon or a nitrogen atom in the moiety Z.

Examples of specific compounds of the present invention include:

6 - [D - α - (4 - aminoquinolin - 3 - carboxamido)phenylacetamido]penicillanic acid;
6 - [D - α - (4 - aminoquinolin - 3 - carboxamido) - 4 - hydroxyphenylacetamido]-penicillanic acid;

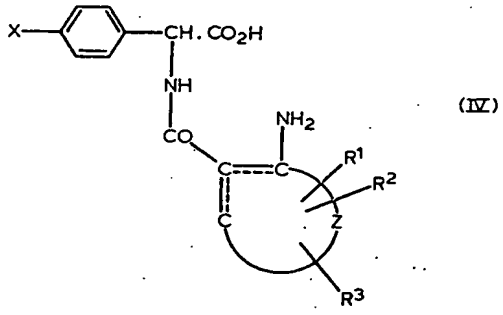
6 - [D - α - (7 - aminopyrazolo[1,5 - a]pyrimidine - 6 - carboxamido]phenylacetamidopenicillanic acid;
6 - [D - α - (7 - aminopyrazolo[1,5 - a]pyrimidine - 6 - carboxamido] - 4 - hydroxyphenylacetamido penicillanic acid;
6 - [D - α - (2 - aminopyridine - 3 - carboxamido)phenylacetamido]penicillanic acid;
6 - [D - α - (2 - aminopyridine - 3 - carboxamido)4 - hydroxyphenylacetamido]penicillanic acid;
6 - [D - α - (4 - amino - 1,5 - naphthridine - 3 - carboxamido)phenylacetamido]penicillanic acid;
6 - [D - α - (4 - amino - 1,5 - naphthridine - 3 - carboxamido) - 4 - hydroxyphenylacetamido]penicillanic acid;
6 - [D - α - (3 - aminopyridazine - 4 - carboxamido)phenylacetamido]penicillanic acid;
6 - [D - α - (3 - aminopyridazine - 4 - carboxamido) - 4 - hydroxyphenylacetamido]penicillanic acid.
6 - [D - α - (4 - amino - 7 - methyl - 1,8 - naphthridine - 3 - carboxamido)phenylacetamido]penicillanic acid;
6 - [D - α - (4 - amino - 7 - methyl - 1,8 - naphthridine - 3 - carboxamido) - 4-hydroxyphenylacetamido]penicillanic acid;
6 - [D - α - (4 - amino - 7 - chloroquinoline - 3 - carboxamido)phenylacetamido]penicillanic acid;
6 - [D - α - (4 - amino - 7 - chloroquinoline - 3 - carboxamido) - 4 - hydroxyphenylacetamido)penicillanic acid.

The compounds of formula (I) may be prepared by reacting a compound of formula (III) or an N-protected derivative which allows acylation to take place thereof:



(III)

wherein R^x is hydrogen an *in vivo* hydrolysable ester radical or a carboxyl blocking group; with an N-acylating derivative of an acid of formula (IV):



(IV)

wherein X, Z, R^1, R^2 and R^3 as defined with respect to formula (I) above and wherein any amino and hydroxy groups may be blocked; and thereafter if necessary carrying out one or more of the following steps:

(i) removal of any N-protecting groups which allow acylation to take place, by hydrolysis or alcoholysis;
(ii) removal of any carboxyl blocking groups;
(iii) removal of any amino or hydroxy blocking groups;
(iv) converting the product to a salt or ester thereof.

Examples of "N-protected derivatives" which allow acylation to take place, of compound (III) include N-silyl and N-phosphorylated derivatives.

By the term "N-silyl derivative" of compound (III), we mean the product of reaction of the 6-amino group of compound (III) with a silylating agent such as a halosilane or a silazane.

Preferred silylating agents are silyl chlorides, particularly trimethylchlorosilane, and dimethyldichlorosilane.

The term "N-phosphorylated" derivative of compound (III) is intended to include compounds wherein the 6-amino group of formula (III) is substituted with a group of formula:

$$-P \cdot R_a R_b$$

wherein R_a is an alkyl, haloalkyl, aryl, aralkyl, alkoxy, haloalkoxy, aryloxy, aralkloxy or dialkylamino group, R_b is the same as R_a or is halogen or R_a and R_b together form a ring.

Suitable carboxyl-blocking derivatives for the group $-CO_2R^x$ in formula (III) include salts and ester derivatives of the carboxylic acid. The derivative is preferably one which may readily be cleaved at a later stage of the reaction. Suitable salts include tertiary amine salts, such as those with tri-loweralkylamines, N-ethylpiperidine, 2,6-lutidine, pyridine, N-methylpyrrolidine, dimethylpiperazine. A preferred salt is with triethylamine.

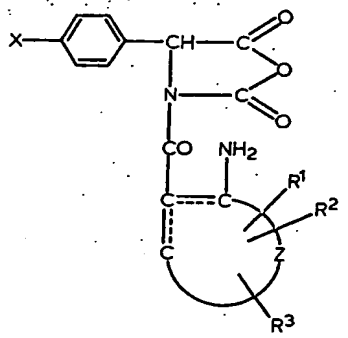
The carboxyl group may be regenerated from any of the above esters by usual methods appropriate to the particular R^x group, for example, acid—and base—catalysed hydrolysis, or by enzymically—catalysed hydrolysis.

A reactive N-acylating derivative of the acid (IV) is employed in the above process. The choice of reactive derivative will of course be influenced by the chemical nature of the substituents of the acid.

Suitable N-acylating derivatives include an acid halide, preferably the acid chloride or bromide. Acylation with an acid halide may be effected in the presence of an acid binding agent for example tertiary amine (such as triethylamine or dimethylaniline), an inorganic base (such as calcium carbonate or sodium bicarbonate) or an oxirane, which binds hydrogen halide liberated in the acylation reaction. The oxirane is preferably a (C_{2-6})-1,2-alkylene oxide—such as ethylene oxide or propylene oxide. The acylation reaction using an acid halide may be carried out at a temperature in the range $-50°$ to $+50°C$, preferably $-20°$ to $+30°C$, in aqueous or non-aqueous media such as aqueous acetone, ethyl acetate, dimethylacetamide, dimethylformamide, acetonitrile, dichloromethane, and 1,2-dichloroethane, or mixtures thereof. Alternatively, the reaction may be carried out in an unstable emulsion of water-immiscible solvent, especially an aliphatic ester of ketone, such as methyl isobutyl ketone or butyl acetate.

The acid halide may be prepared by reacting the acid (IV) or a salt thereof with a halogenating (e.g. chlorinating or brominating) agent such as phosphorus pentachloride, thionyl chloride or oxalyl chloride.

Alternatively, the N-acylating derivative of the acid (IV) may be a symmetrical or mixed anhydride. Suitable mixed anhydrides are alkoxyformic anhydrides, or anhydrides with, for example carbonic acid monoesters, trimethyl acetic acid, thioacetic acid, diphenylacetic acid, benzoic acid, phosphorus acids (such as phosphoric or phosphorous acids), sulphuric acid or aliphatic or aromatic sulphonic acids (such as *p*-toluenesulphonic acid). The mixed or symmetrical anhydrides may be generated *in situ*. For example, a mixed anhydride may be generated using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. When a symmetrical anhydride is employed, the reaction may be carried out in the presence of 2,4-lutidine as catalyst. Another type of anhydride is the 2,5-oxazolidinedione of formula (V):



wherein X, Z, R^1, R^2 and R^3 are as defined with respect to formula (I) above. Com-

pound (V) may be prepared from the acid (IV) by the action of phosgene.

Alternative N-acylating derivatives of acid (IV) are the acid azide, or activated esters such as esters with 2-mercaptopyridine, cyanomethanol, *p*-nitrophenol, 2,4-dinitrophenol, thiophenol, halophenol, including pentachlorophenol, monomethoxyphenol or 8-hydroxyquinoline; or amides such as N-acylsaccharins or N-acylphthalimides; or an alkylidene iminoester prepared by reaction of the acid (IV) with an oxime.

Some activated esters, for example the ester formed with 1-hydroxybenztriazole or N-hydroxysuccinimide, may be prepared *in situ* by the reaction of the acid with the appropriate hydroxy compound in the presence of a carbodiimide, preferably dicyclohexylcarbodiimide.

Other reactive N-acylating derivatives of the acid (IV) include the reactive intermediate formed by reaction *in situ* with a condensing agent such as a carbodiimide, for example, N,N-diethyl-, dipropyl- or diisopropylcarbodiimide, N,N′-cyclohexylcarbodiimide, or N-ethyl-N′-γ-dimethylaminopropylcarbodiimide; a suitable carbonyl compound, for example N,N′-carbonyldiimidazole or N,N′-carbonylditriazole; and isoxazolinium salt, for example N - ethyl - 5 - phenylisoxazolinium - 3 - sulphonate or N - *t* - butyl - 5 - methylisoxazolinium perchlorate; or an N - alkoxycarbonyl - 2-alkoxy - 1,2 - dihydroquinoline, such as N - ethoxycarbonyl - 2 - ethoxy - 1,2 - dihydroquinoline. Other condensing agents include Lewis acids (for example BBr_3—C_6H_6); or a phosphoric acid condensing agent such as diethylphosphorylcyanide. The condensation reaction is preferably carried out in an organic reaction medium, for example methylene chloride, dimethylformamide, acetonitrile, alcohol, benzene, dioxan, or tetrahydrofuran.

In the above process, when any of the groups X, R^1, R^2 and R^3 represent a hydroxygroup, it may be protected prior to the acylation reaction by known methods, for example by esterification or acylation. In general however, hydroxyl protection is not required.

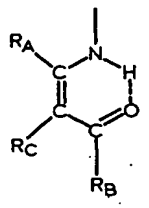
The amino substituent on the heterocyclic ring in formula (IV) may also be blocked. By a blocked amino group is meant an amino group substituted with a group which can be removed after the acylation reaction under conditions sufficiently mild to avoid destruction of the molecule; or a group which can be converted to an amino group, again under mild conditions.

Examples of blocked amino groups include the protenated amino group (NH_3^+) which after the acylation reaction can be converted to the free amino group by simple neutralisation; and the β,β,β-trichloroethoxycarbonylamino radical which may be converted to amino by reduction with zinc in acetic acid.

Blocked amino groups which regenerate the amino group by catalytic hydrogenation include benzyloxycarbonylamino; p-substituted benzyloxycarbonylamino where the substituent is halogen (especially chlorine), nitro, or methoxy; triphenylmethyl; azido or nitro. The hydrogenation is preferably carried out at room temperature and either at atmospheric or slightly elevated pressure. Preferred catalysts are noble metal catalysts for instance palladium or platinum, or Raney-nickel. Reduction of these groups may also be effected by electrolytic reduction.

Groups which regenerate the amino group on mild acid hydrolysis include the *tert*-butyloxycarbonylamino group which may be converted to amino by treatment with trifluoroacetic acid, hydrogen chloride, or *p*-toluenesulphonic acid.

Another example of a blocked amino group which may be subsequently converted to amino by mild acid hydrolysis is a group of formula:

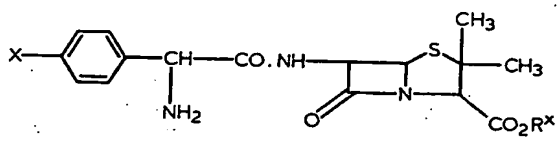


wherein R_A is an alkyl, aralkyl, or aryl group, R_B is an alkyl, aralkyl, aryl, alkoxy, aralkoxy or aryloxy group, and R_C is a hydrogen atom or an alkyl, aralkyl, or aryl group, or R_C together with either R_A or R_B completes a carbocyclic ring.

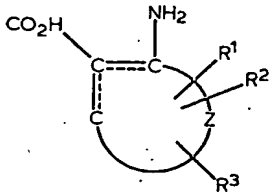
Other blocked amino groups include bromine which may be converted by amination, for instance with hexamethylenetetramine; o-nitrophenylsulphenylamino which

may be converted to amino by reaction with sodium or potassium iodide sodium thiosulphate, sodium hydrosulphide, sodium hydrosulphite, or potassium thiocyanate.

The compounds of formula (I) may also be prepared by reaction of a compound of formula (XI) or an N-protected derivative which allows acylation to take place, thereof:



wherein X is as defined with respect to formula (I) and R^x is a carboxyl blocking group; with an N-acylating derivative of an acid of formula (IX):



(IX)

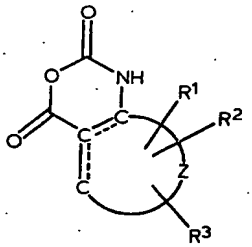
wherein Z, R^1, R^2, and R^3 are as defined with respect to formula (I) above and wherein the amino and any hydroxy groups may be blocked; and thereafter if necessary carrying out one or more of the following steps:

(i) removal of any N-protecting groups which allow acylation to take place, by hydrolysis or alcoholysis;
(ii) removal of any carboxyl blocking groups;
(iii) removal of any amino or hydroxy blocking groups;
(iv) converting the product to a salt or ester thereof.

The comments made earlier concerning N-protected derivatives which allow acylation to take place, blocking groups and N-acylating derivatives also apply to this process.

In particular a preferred blocked amino group is the azide group. Alternatively an N-acylating derivative of an acid (IX) may also be employed with the free amino group.

A preferred N-acylating derivative of the acid (IX) is the anhydride (X):



(X)

wherein Z, R^1, R^2 and R^3 are as defined with respect to formula (I).

The antibiotic compounds according to the invention may be formulated for administration in any convenient way for use in human and veterinary medicine, by analogy with other antibiotics, and the invention therefore includes within its scope a pharmaceutical composition comprising a compound of formula (I) above together with a pharmaceutical carrier or excipient.

The compositions may be formulated for administration by any route. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

Tablets and capsules for oral administration may be in unit dose presentation

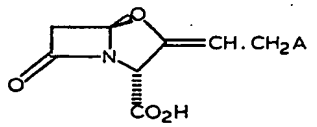
form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine, tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl *p*-hydroxybenzoate or sorbic acid, and if desired convention flavouring or colouring agents.

Suppositories will contain conventional suppository bases e.g. cocoa, butter or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial. Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The compound can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

The compositions may contain from 0.1% to 99% by weight, preferably from 10—60% by weight, of the active material, depending on the method of administration. Where the compositions comprise dosage units, each unit will preferably contain from 50—500 mg., of the active ingredient. The dosage as employed for adult human treatment will preferably range from 100 to 3000 mg., per day, for instance 1500 mg., per day, depending on the route and frequency of administration.

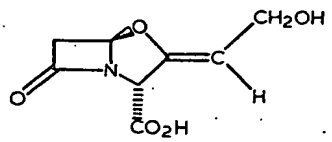
The compound of formula (I) may be the sole therapeutic agent in the compositions of the invention or a combination with other antibiotics may be employed. Advantageously the compositions also comprise a compound of formula (XIII) or a pharmaceutically acceptable salt or ester thereof:



(XIII)

wherein A is hydrogen or hydroxyl.

Preferably the compound of formula (XIII) is clavulanic acid of formula (XIV) or a pharmaceutically acceptable salt or ester thereof:



(XIV)

The preparation of these compounds is described in Belgium Patent nos. 827,926, 836,652 and West German Offenlegungsschrift no. 2,616,088.

It will be clear that the side-chain of the penicillins of formula (I) contains a

potentially asymmetric carbon atom. This invention includes all the possible epimers of compounds (I) as well as mixtures of them.

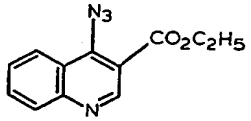
The following examples illustrate the preparation of some of the compounds of this invention.

The following literature references are referred to in the Examples:

1. B. Riegel *et al*, J. Amer. Chem. Soc. 1946, *68*, 1265.
2. Makisami *et al*, Chem. Pharm. Bull, 10(7), 620—6 (1962).
3. A. L. J. Beckwith and R. J. Hickman, J. Chem. Soc. (C) 2756 (1968).

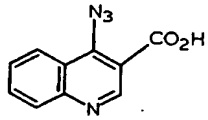
Example 1.

(a) *4-Azido-3-carbethoxyquinoline*



3-Carbethoxy-4-chloroquinoline[1] (1.8 g; 0.0076M) was dissolved in dry DMF (15 ml) at ambient temperatures and sodium azide (0.8g; 0.012M) added. The mixture was stirred at ambient temperatures for 24 hr. A large volume of Et_2O (250ml) was added followed by H_2O (25ml) and the layers separated. The aqueous phase was further extracted with Et_2O (2×25ml), the Et_2O extracts combined, washed well with saturated brine, dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to yield a white solid, 1.77g (96%), m.p. 52—53°C (Found: C; 59.67; H, 4.13; N, 23.22%; $C_{12}H_{10}N_4O_2$ requires C, 59.50; H, 4.13; N, 23.14%) ν_{max} (KBr) 2130, 1712, 1583, 1494, 1390, 1378, 1322, 1240, 857cm^{-1} δ [$(CD_3)_2SO$] 1.4(t), 4.49(q) (CH_3CH_2), 7.56—8.5(m) 9.15(s) (aromatic protons), *m/e* 242(M^+).

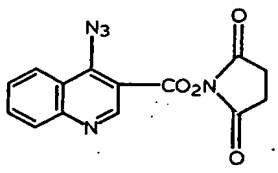
(b) *4-Azido-3-quinolinic acid*



4-Azido-3-carbethoxyquinoline (1.4g; 0.006M) was suspended in 10% aq. NaOH at ambient temperatures and the mixture stirred until complete solution had been obtained. The solution was filtered, cooled to 0°C and acidified to pH 4 with 5M HCl. The resulting precipitate was filtered, washed well with H_2O and dried *in vacuo* over P_2O_5 to yield the product, 1.3g (93%), as a monohydrate, m.p. 284°C(dec.) (Found: N, 24.53%, $C_{10}H_8N_4O_3$ requires: N, 24.46%), ν_{max} (nujol) (Registered Trade Mark) 3200—3700(br), 2200—2600(br), 2110, 1700, 1492, 1327, 1215, 760cm^{-1}, δ[$(CD_3)_2SO$] 7.45—8.1(m), 8.9(s) (aromatic protons), 14—16 (broad) ($CO_2H^*+H_2^*O$).

* Exchangeable with D_2O.

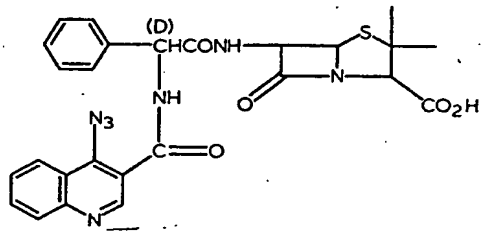
(c) *N-[4-Azido-3-quinolinoyloxy]succinimide*



4-Azido-3-quinolinic acid monohydrate (2.3g; 0.01M) was suspended at ambient temperatures in dry DMF (25ml). N-hydroxysuccinimide (1.2g; 0.01M) was added and the resulting mixture cooled to 0—5°C. N,N-dicyclohexylcarbodiimide (2.3g; 0.11M) was added and the mixture stirred at 0—5°C for ½hr. then at ambient temperatures for 4 days. The insoluble material was removed by filtration and the filtrate evaporated to dryness *in vacuo*. The residual solid was recrystallised from iso-propyl alcohol as a light-brown, crystalline solid, 2.5g (80%), m.p. 173—5°C(dec.) (Found:

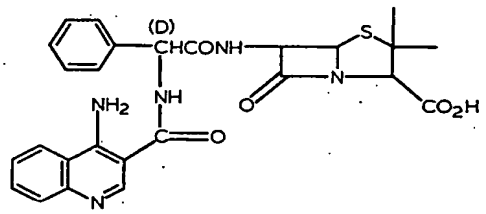
C, 53.71; H, 2.80; N, 22.47%. $C_{14}H_9N_5O_4$ requires: C, 54.02; H, 2.89; N, 22.47%), ν_{max} (KBr), 2120, 1790, 1760, 1730, 1490, 1390, 1370, 1202, 890, 780, 640cm^{-1}, δ[$(CD_3)_2SO$] 2.9(s)(CH_2CH_2), 7.57—8.4(m), 9.18(s) (aromatic protons), *m/e* 311(M^+).

(d) *6 - [D - α - (4 - Azidoquinoline - 3 - carboxamido)phenylacetamido]penicillanic acid*



N - (4 - Azido - 3 - quinolinoyloxy)succinimide (1.6g; 0.005M) was dissolved in acetone (250ml) and added to H_2O (100ml) containing sodium 6-(D-α-aminophenylacetamido)penicillanate (1.9g; 0.005M). The mixture was stirred at ambient temperatures for 3 hrs before the acetone was removed *in vacuo*. The insoluble material, 0.83g; m.p. 166—68°C (dec.), was filtered, washed with H_2O and dried in air and shown by I.R. spectroscopy to be recovered 'activated' ester. The filtrate was acidified to pH 2.5 with 5M HCl and the product, 0.6 g (57%), filtered, washed with H_2O and dried over P_2O_5 *in vacuo* ν_{max} (KBr) 3100—3700(br), 2122, 1780, 1735, 1650, 1495, 1380, 1300, 1220, 770, 700cm^{-1}, δ[$(CD_3)_2SO$] 1.42(s), 1.56(s) (gem dimethyls), 4.22(s) (C_3 proton), 5.37—5.7(m)(β-lactams), 6.05(d)(α-proton), 7.2—8.4(m), 8.88(s) (aromatic+heteroaromatic protons), 9.2(d), 9.68(d) (2× CONH*), CO_2H* diffuse, low field resonance, *exchangeable with D_2O. biochromatogram, Rf (B/E/W)≃0.80 (single zone).

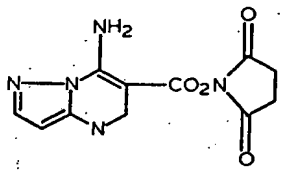
(e) *6 -[D - α - (4 - Aminoquinoline - 3 - carboxamido)phenylacetamido]penicillanic acid*



5% $Pd/CaCO_3$ (0.1 g) was suspended in H_2O (10ml) and hydrogenated at ambient temperatures and atmospheric pressure for 1 hour. After 1 hour a solution in H_2O (10ml) of 6 - [D - α - (4 - Aminoquinoline - 3 - carboxamido)phenylacetamido]penicillanic acid (0.1g; 0.00018M) and $NaHCO_3$ (0.016g; 0.00018M) was added and the mixture hydrogenated at ambient temperatures and atmospheric pressure for 1 hour. The reaction mixture was filtered through Kieselgühr and the filtrate acidified to pH 2.5 with 5M HCl to precipitate the product, 80mg (86%), ν_{max} (nujol) (Registered Trade Mark) 3300(br), 1763, 1640, 1610, 1520(br), 1320, 770, 735, 705cm^{-1}, δ[$(CD_3)_2SO$] 1.39(s), 1.49(s) (gem dimethyls), 4.15(s)(C_3 proton), 5.3—5.6(m) (β-lactams), 5.84(d) (α-proton), 7.4—7.9(m), 8.2—8.7(m), 8.72—9.1(br)(aromatics+heteroaromatics+2×CONH*) NH_2* and CO_2H* broad, diffuse low field resonances, *exchangeable with D_2O, biochromatogram Rf(B/E/W)≃0.7 (single zone).

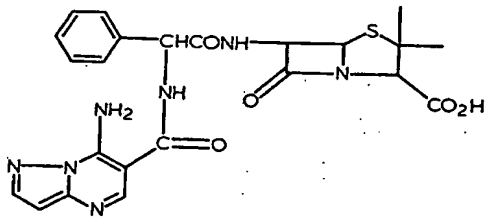
Example 2.

(a) *N - [7 - Aminopyrazolo[1,5 - a]pyrimidine - 6 - carbonyloxy]succinimide*



7 - Aminopyrazolo[1,5 - a]pyrimidine - 6 - carboxylic acid[2] (0.18g; 0.001M) was suspended in dry D.M.F. (15ml) and the mixture stirred and cooled at 0—5°C. N-hydroxysuccinimide (0.13g; 0.0011M) was added followed by $SOCl_2$ (0.15g; 0.0013M), which was added dropwise. After 15 min. at 0—5°C, $SOCl_2$ (0.15g; 0.0013M) was again added dropwise and a clear solution was obtained. The reaction was stirred at 0—5°C for ½hr. then at ambient temperatures for 24hr. After 24hr., the reaction mixture was cooled to 0—5°C and pyridine (0.42g; 0.006M) added dropwise. Stirred at 0—5°C for 1 hr., then at ambient temperatures for 4hr. The reaction mixture was kept at 0°C overnight and the solvent removed *in vacuo*. The product was precipitated from solution at low volume by addition of H_2O and collected, washed well with H_2O and dried over P_2O_5 *in vacuo*, 0.146g. (53%), m.p. 294—6°C (dec.), ν_{max} (nujol) (Registered Trade Mark) 3040, 1785, 1737(br), 1680, 1615, 1580, 1455, 1445, 1350, 1290, 1198, 1060cm^{-1}, δ[$(CD_3)_2SO$] 2.8(s)(CH_2CH_2), 6.33(d) 7.9(d), 8.73(s) (heteroaromatic protons), NH_2* broad, diffuse low-field resonance, *exchangeable with D_2O, m/e 275(M^+).

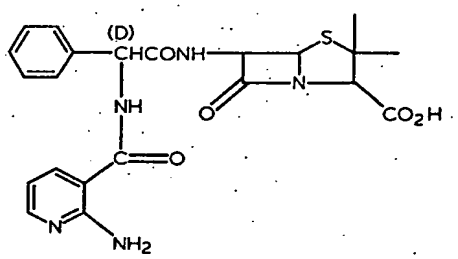
(b) *6 - [D - α - (7 - Aminopyrazolo[1,5 - a]pyrimidine - 6 - carboxamido]phenylacetamido]penicillanic acid*



N - [7 - Aminopyrazole[1,5 - a]pyrimidine - 6 - carbonyloxy]succinimide (0.213g; 0.0008M) was suspended in dry D.M.F. (6ml) at ambient temperatures and with vigorous stirring. Sodium 6-(D-α-aminophenylacetamido)penicillanate (0.28g; 0.0008M) dissolved in dry D.M.F. (2ml) was added and the mixture stirred at ambient temperatures for 1½hr. The reaction mixture was added slowly to a large volume of rapidly-stirred, dry Et_2O and the resulting precipitate was filtered off, washed well with dry Et_2O and redissolved in H_2O (min. volume). The aqueous solution was filtered and the filtrate acidified to pH 2.5 with 5M HCl and the resulting precipitate, 0.104g (25%), collected, washed well with H_2O and dried over P_2O_5 *in vacuo*, ν_{max} (KBr) 3600—3100(br), 3040, 1770, 1725, 1670(br), 1620, 1582, 1520(br), 1460, 1300, 1210, 789, 700cm^{-1}, δ[$(CD_3)_2SO$] 1.45(s), 1.59(s) (gem dimethyls), 4.25(s) (C_3 proton), 5.35—5.73(m) (β-lactams), 6.0(d) (α-proton), 6.4(d), 8.05(d), 8.67(s) (heterocyclic protons), 7.4(br)(aromatic protons), 9.3(d), 9.9(d) (2×CONH*), NH_2* diffuse between 5.3 and 6.9, CO_2H* diffuse, low-field resonance, *exchangeable with D_2O, biochromatogram, Rf (B/E/W) ≏0.3 (single zone), hydroxylamine assay 75% (v. Pen G.)

Example 3.

6 - [D - α - (2 - Aminopyridine - 3 - carboxamido)phenylacetamido]penicillanic acid

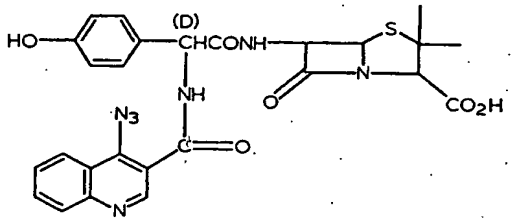


Sodium 6 - (D - α - aminophenylacetamido)penicillanate (1.8g; 0.0048M) was dissolved in H_2O (20ml.) at ambient temperatures with stirring and 2,4 - dihydro-2,4 - dioxo - 1 - H - pyrido[2,3 - d][1,3]oxazine[5] added. The mixture was stirred at ambient temperatures for 1hr. and the insoluble material removed by filtration, m.p. 212—213°C(dec.). This was shown by i.r. spectroscopy to be recovered 2,4-dihydro-2,4-dioxo-1-H-pyrido[2,3-d][1,3]oxazine.

The filtrate was cooled to 0—5°C and acidified to pH 2.6 with 5M HCl and the precipitate collected by filtration, washed well with H_2O and dried over P_2O_5 *in vacuo*, 0.4g (18%), ν_{max} (KBr) 3700—3100(br), 1770, 1700—1600(br), 1570, 1500, 1315, 1250, 770, 700cm^{-1}, δ[$(CD_3)_2SO$] 1.41(s), 1.52(s), (gem dimethyls), 4.22(s) (C_3 proton), 5.3—5.7(m)(β-lactams), 5.87(d)(α-proton), 6.45—6.79(m), 7.2—7.7(br), 7.95—8.2(m) (aromatic+heteroaromatic protons), 6.79—7.2(br) (NH_2*), 8.8(d), 9.03(d) (2×CONH*), CO_2H* diffuse, low-field resonance, biochromatogram, Rf (B/E/W)≏0.53 (single zone), hydroxylamine assay 93% (v. Pen.G).

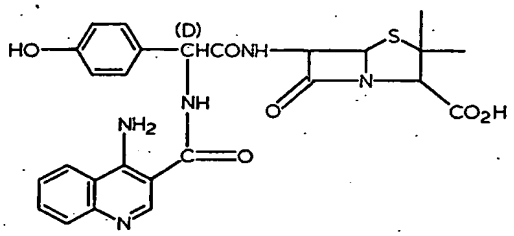
Example 4.

a) *6 - [D - α - (4 - Azidoquinoline - 3 - carboxamido) - α - (4 - hydroxyphenyl)-acetamido]penicillanic acid*



N - (4 - Azido - 3 - quinolinoyloxy)succinimide (0.7g; 0.0022M) was dissolved with stirring at 0—5°C in the min., dry D.M.F. (20ml). Triethylammonium 6 - (D-α - amino - α - (4 - hydroxyphenyl)acetamido)penicillanate (1.05g; 0.0022M) was added and the mixture stirred at 0—5°C for 1 hr. then allowed to regain ambient temperatures over ½hr. The reaction mixture was poured carefully into rapidly-stirred, dry Et_2O (2l) and the precipitate removed by filtration, carefully washed with dry Et_2O and immediately redissolved in H_2O (50ml). The aqueous mixture was filtered and the pH adjusted to 2.5 with 5M HCl. The product was filtered off, washed well with H_2O and dried over P_2O_5 *in vacuo* (0.8g; 65%), ν_{max} (KBr) 3700—3100 (br), 2138, 1775, 1740, 1645 (br), 1618, 1519, 1380, 1227, 770cm^{-1}, δ[$(CD_3)_2SO$] 1.4(s), 1.52(s) (gem dimethyls), 4.27(s) (C_3 proton), 5.4—5.8 (m) (β-lactams), 5.94(d) (α-proton), 6.79(d), 7.4 (d) (p—HO—C_6H_4—), 7.6—8.4 (m), 8.9 (s) (heterocyclic protons), 9.06 (d), 9.59 (d) (2×CONH*), OH* and CO_2H* diffuse, low-field resonances, *exchangeable with D_2O, biochromatogram, Rf (B/E/W)≏0.68, hydroxylamine assay 97.7% (versus Pen.G)

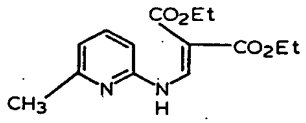
b) *6 - [D - α - (4 - Aminoquinoline - 3 - carboxamido) - α - (4 - hydroxyphenyl)-acetamido]penicillanic acid (AB 20196)*



6 - [D - α - (4 - Azido - 3 - quinolinamido) - α - (4 - hydroxyphenyl)acetamido]penicillanic acid (0.4g; 0.0007M) was suspended in H_2O (25ml) and the mixture stirred at ambient temperatures. $NaHCO_3$ (0.06g; 0.007M) was added and the mixture stirred until complete solution had been obtained. This solution was added to a suspension of 5% Pd/$CaCO_3$ in H_2O (10ml) which had been pre-hydrogenated for 1hr. at atmospheric pressure and ambient temperatures. This mixture was then hydrogenated for 1¼hr. at atmospheric pressure and ambient temperatures before the catalyst was removed by filtration through Kieselgühr and the filtrate acidified to pH 2.8 with *5M* HCl and the product (0.3; 75%) collected by filtration, washed with cold H_2O and dried over P_2O_5 *in vacuo*, ν_{max} (KBr) 3700—2300 (br), 1768, 1640 (br), 1610, 1510, 1380, 1320, 1250, 770cm^{-1}, δ[$(CD_3)_2SO$] 1.40(s), 1.50(s) (gem dimethyls), 4.17(s) (C_3 proton), 4.5—5.9 (br) ($3\times H_2O$*), 5.3—5.7 (m) (β-lactams), 5.73 (d) (α-proton), 6.72 (d), 7.31 (d) (p—HO—C_6H_4—), 7.4—9.1 (m) (heterocyclic protons+$2\times$COHN*+NH_3^+*), OH* diffuse, low-field resonance, *exchangeable with D_2O, biochromatogram, Rf (B/E/W)≃0.58, hydroxylamine assay 102.0% (versus Pen G.).

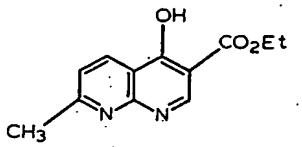
Example 5.

a) *2-(2,2-Dicarbethoxy-1-vinylamino)-6-methylpyridine*



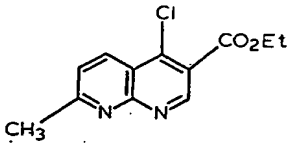
2-Amino-6-methylpyridine (108g; 1M) and diethyl ethoxymethylenemalonate (216g; 1M) were mixed together and refluxed for 2hr. in EtOH (250 ml.). The reaction mixture was left at ambient temperatures overnight and the product was filtered off, washed with EtOH and dried *in vacuo* over P_2O_5 (249.7g; 90%), m.p. 107—8°C.

b) *3 - Carbethoxy - 4 - hydroxy - 7 - methyl - 1,8 - naphthyridine*



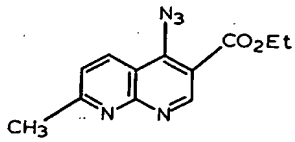
2 - (2,2 - Dicarbethoxy - 1 - vinylamino) - 6 - methylpyridine (47.2g; 0.17M) was added to vigorously refluxing diphenyl ether (300ml) and the mixture refluxed 20 min. The reaction mixture was allowed to regain ambient temperatures and the product removed by filtration, washed well with petroleum ether (40—60°C), dissolved in boiling MeOH and the solution was decolourised by refluxing with for ½hr. The charcoal was removed by filtration through Kieselgühr and the MeOH removed *in vacuo* to dryness. The residual yellow solid was stirred in $CHCl_3$ and the product filtered off, washed with $CHCl_3$ and dried in air, 10.5g (26%), m.p. 270—271°C (dec.).

c) *3 - Carbethoxy - 4 - chloro - 7 - methyl - 1,8 - naphthyridine*



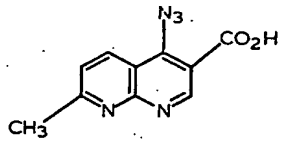
3 - Carbethoxy - 4 - hydroxy -7 - methyl - 1,8 - naphthyridine (3.8g; 0.016M) was suspended in $POCl_3$ (46ml; 0.45M) and the mixture heated at 70—80°C for 4hr. The solution was concentrated *in vacuo* and the residue poured carefully onto crushed ice. The resulting solution was basified with 10% aq. NaOH to pH 6 and extracted with Et_2O. The Et_2O extracts were combined, washed with saturated brine and dried over anhydrous $MgSO_4$. The drying agent was removed by filtration and the filtrate was decolourised by refluxing with charcoal, filtered through Kieselgühr and evaporated to dryness *in vacuo* to yield the product, 3.7g. (92%), m.p. 92—93°C (dec.). An analytical sample was obtained by chromatography over silica gel using $CHCl_3$/MeOH (9:1) as eluent. m.p. 90—91°C (dec.) (Found: N, 11.36; C, 57.80; H, 4.66; Cl, 14.13%. $C_{12}H_{11}ClN_2O_2$ requires: N, 11.18; C,57.48; H, 4.39; Cl, 14.17%), ν_{max} (KBr) 1720, 1600, 1580, 1470, 1260, 1213, 1170, 1022, 810cm^{-1}, δ[$(CD_3)_2SO$] 1.39(t), 4.4 (q), (CH_3CH_2), 2.74 (s) (CH_3), 7.69 (d), 8.6 (d), 9.21 (s) (heterocyclic protons), m/e 250 (M^+; 100%), 222 (41%).

d) *4 - Azido - 3 - carbethoxy - 7 - methyl - 1,8 - naphthyridine*



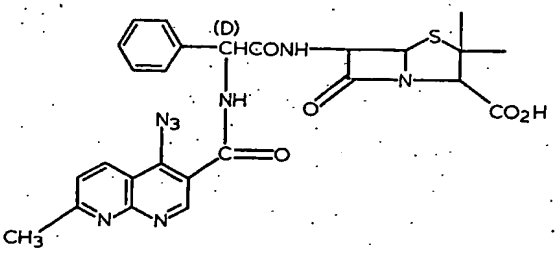
3 - Carbethoxy - 4 - chloro - 7 - methyl - 1,8 - naphthyridine (0.8g; 0.003M) was dissolved in dry D.M.F. (5ml) at ambient temperatures and NaN_3 (0.5g; 0.007M) added. This mixture was stirred for 20hr. at ambient temperatures and then poured into a large volume (1l) of H2O. The product (0.64g; 83%), m.p. 114—115°C (dec.), was filtered off, washed with H_2O and dried over P_2O_5 *in vacuo* (Found: N, 27.49; C, 55.59; H, 4.50%. $C_{12}H_{11}N_5O_2$ requires: N, 27.24; C, 56.03; H, 4.28%), ν_{max} (KBr) 3080, 2900, 2142, 1708, 1600, 1550, 1472, 1375, 1268, 1210, 1194, 1050, 1038, 806cm^{-1}, δ[$(CD_3)_2SO$] 1.38 (t), 4.40 (q), (CH_3CH_2), 2.68 (s), (CH_3), 7.5 (d), 8.5 (d), 9.14 (s) (heterocyclic protons), m/e 257 (M^+; 65%) 229 (M^+—N_2; 32%), 212 (M^+—OC_2H_5; 15%), 201 (52%), 133 (100%).

e) *4 - Azido - 7 - methyl - 1,8 - naphthyridine - 3 - carboxylic acid*



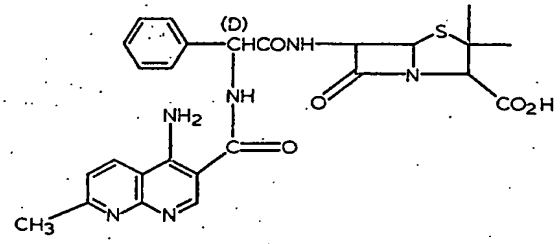
4 - Azido - 3 - carbethoxy - 7 - methyl - 1,8 - naphthyridine (2.4g; 0.008M) was suspended in 10% aq. NaOH (60ml) and the mixture stirred at ambient temperatures until all the ester had reacted. The insoluble material was removed by filtration and redissolved in H_2O. The pH of the solution was adjusted to 3.5 with *5M* HCl and the resulting precipitate (1.3g; 71%) filtered off, washed with H_2O and dried over P_2O_5 *in vacuo*, m.p. 198° C (explosive dec. on rapid heating), (Found: N, 29.66%. $C_{10}H_7N_5O_2 \cdot \frac{1}{2}H_2O$ requires N, 29.41%), m/e 201 (M^+—N_2; 95%), 159 (100%) ν_{max} (KBr) 3430 (br), 2430 (br), 2150, 2060—1800 (br), 1705, 1605, 1560, 1475, 1375, 1260, 1230, 1202, 920, 810cm^{-1}, δ [$(CD_3)_2SO$] 2.72 (s) (CH_3), 7.62 (d), 8.62 (d), 9.29 (s) (heterocyclic protons), CO_2H* diffuse low-field resonance, *exchangeable with D_2O.

f) *6 - [D - α - (4 - Azido - 7 - methyl - 1,8 - naphthyridine - 3 - carboxamido)-phenylacetamido]penicillanic acid.*



4 - Azido - 7 - methyl - 1,8 - naphthyridine - 3 - carboxylic acid hemihydrate (1.19g; 0.005M) was suspended in dry D.M.F. (50ml) at 0—5° C and the mixture rapidly stirred. N-hydroxysuccinimide (0.6 g; 0.005M) and N,N' - dicyclohexylcarbodiimide (1.13g; 0.0055M) were added and the reaction mixture stirred at 0—5° C for 1hr. then at ambient temperatures for 4 days. The reaction mixture was cooled to 0—5° C again and sodium 6 - (D - α - aminophenylacetamido)penicillanate (1.8g; 0.005M) added. This mixture was stirred at 0—5° C for 1hr. and allowed to regain ambient temperatures over ½hr. The mixture was filtered into rapidly-stirred, dry Et_2O (2l) and the resulting precipitate filtered off, washed well with dry Et_2O and immediately redissolved in H_2O (50ml). The aqueous mixture was filtered and the pH adjusted to 2.5 with *5M* HCl. The product (0.8g; 32%) was collected by filtration, washed with H_2O and dried over P_2O_5 *in vacuo*, ν_{max} (KBr) 3700—3100 (br), 2140, 1775, 1738, 1650 (br), 1602, 1520 (br), 1380, 1350—1250 (br), 1225, 808, 702cm^{-1}, δ [$(CD_3)_2SO$] 1.43 (s), 1.57 (s) (gem dimethyls), 2.71 (s) (CH_3), 4.23 (s) (C_3 proton) 5.3—5.7 (m) (β-lactams). 6.03 (s) (α-proton), 7.2—7.8 (m), 8.55 (d), 9.01 (s) (aromatic + heteroaromatic protons), 9.2 (d), 9.7 (d) (2 × CONH*), CO_2H* diffuse, low-field resonance, *exchangeable with D_2O, biochromatogram, Rf (B/E/W). ≏ 0.43, hydroxylamine assay 98.7% (versus Pen G.).

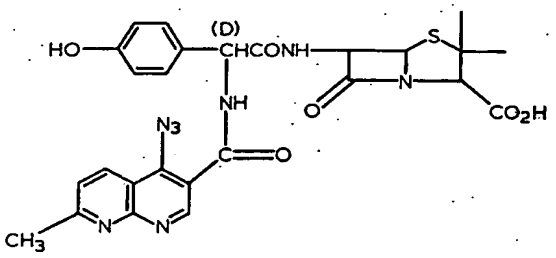
g) *6 - [D - α - (4 - Amino - 7 - methyl - 1,8 - naphthyridine - 3 - carboxamido)-phenylacetamido]penicillanic acid*



6 - [D - α - (4 - Azido - 7 - methyl - 1.8 - naphthyridine - 3 - carboxamido)-phenylacetamido]penicillanic acid (0.56g; 0.001M) was dissolved in H_2O (20ml) containing $NaHCO_3$ (0.084g; 0.001M). This solution was added to a suspension of 5% Pd/$CaCO_3$ (0.56g) in H_2O (10ml), which had been pre-hydrogenated for 1hr. at ambient temperatures and atmospheric pressure. This mixture was hydrogenated at atmospheric pressure and ambient temperatures for 1¼hr., the catalyst removed by filtration through Kieselguhr and the filtrate acidified to pH 4 with *5M* HCl. The product (0.26g; 49%) was collected by filtration, washed with H_2O and dried over P_2O_5 *in vacuo*, ν_{max} (KBr) 3700—2200 (br), 1765, 1700—1550 (br), 1515, 1460, 1370, 1325, 1260, 1220, 1080, 800, 702cm^{-1}, δ [$(CD_3)_2SO$] 1.41 (s), 1.51 (s) (gem dimethyls), 2.61 (s) (CH_3) 4.18 (s) (C_3 proton), 5.2—5,6 (m) (β-lactams), 5.9 (d) (α proton), 5.6—6.7 (br), (3 × H_2O*), 7.0—7.8 (m), 8.1—9.3 (broad m) (aromatics + heteroaromatics + NH_3^{+*} + 2 × CONH*), *exchangeable with D_2O, biochromatogram, Rf (B/E/W) ≏ 0.53, hydroxylamine assay 87% (versus Pen G.).

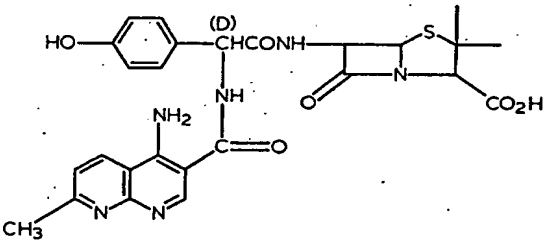
Example 6.

a) *6 - [D - α - (4 - Azido - 7 - methyl - 1,8 - naphthyridine - 3 - carboxamido)- α - (4 - hydroxyphenyl)acetamido]penicillanic acid*



4 - Azido - 7 - methyl - 1,8 - naphthyridine - 3 - carboxylic acid (1.15g; 0.005M) was suspended in dry D.M.F. (50ml) at 0—5°C with stirring and N-hydroxy - succinimide (0.6g; 0.005M) and N,N' - dicyclohexylcarbodiimide (1.13g; 0.0055M) were added. The mixture was stirred at 0—5°C for 1hr. and allowed to regain ambient temperatures before being stirred at ambient temperatures for 4 days. The reaction mixture was re-cooled to 0—5°C and triethylammonium 6 - [D - α-amino - α - (4 - hydroxyphenyl)acetamido]penicillanate (2.3g; 0.0049M) added. The reaction mixture was then stirred at 0—5°C for 1hr. and then allowed to regain ambient temperatures over ½hr. before being filtered into rapidly-stirred, dry Et_2O (2l). The precipitate was filtered off, washed with dry Et_2O and immediately redissolved in H_2O (50ml), the aqueous mixture filtered and the pH of the filtrate adjusted to 3 with 5M HCl and the product (0.86g; 30%) collected by filtration, washed with H_2O and dried over P_2O_5 *in vacuo*, ν_{max} (KBr) 3700—3100 (br), 2140, 1770, 1733, 1650 (br), 1601, 1510, 1380, 1270, 1230 (br), 840, 808cm^{-1}, δ [$(CD_3)_2SO$] 1.46 (s), 1.60 (s) (gem dimethyls), 2.73 (s) (CH_3), 4.22 (s) (C_3 proton), 5.38—5.75 (m) (β-lactams), 5.9 (d) (α-proton), 6.79 (d), 7.4 (d) (p-HO—C_6H_4—), 7.6 (d), 8.54 (d), 9.0 (s) (heteroaromatic protons), 9.06 (d), 9.59 (d) (2 × CONH*), CO_2H* and OH* diffuse, low-field resonances, *exchangeable with D_2O, biochromatogram, Rf (B/E/W) ≙ 0.54, hydroxylamine assay 84.5% (versus Pen G.).

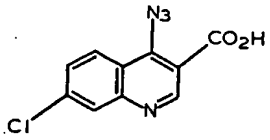
b) *6 - [D - α - (4 - Amino - 7 - methyl - 1,8 - naphthyridine - 3 - carboxamido)- α - (4 - hydroxyphenyl)acetamido]penicillanic acid.*



6 - [D - α - (4 - Azido - 7 - methyl - 1,8 - naphthyridine - 3 -carboxamido)-α-(4-hydroxyphenyl)acetamido]penicillanic acid (0.8g; 0.0014M) was dissolved in H_2O (25ml) containing $NaHCO_3$ (0.12g; 0.0014M) and the solution added to a suspension of 5% $Pd/CaCO_3$ (0.8g) in H_2O (10ml), which had been pre-hydrogenated for 1hr. at ambient temperatures and atmospheric pressure. The resulting mixture was hydrogenated 1¾hr. at ambient temperatures and atmospheric pressure before the catalyst was removed by filtration through Kieselgühr. The pH of the filtrate was adjusted to 3.5 with 5M HCl and the product (0.4g; 47%) removed by filtration, washed with H_2O and dried over P_2O_5 *in vacuo*, ν_{max} (KBr) 3700—2250 (br), 1765, 1700—1550 (br), 1510, 1460, 1370, 1325, 1265, 1245, 800cm^{-1}, δ [$(CD_3)_2SO$] 1.41 (s), 1.50 (s) (gem dimethyls), 2.60 (s) (CH_3), 4.15 (s) (C_3 proton), 5.3—5.6 (m) (β-lactams), 5.7 (d) (α-proton), 5.8—6.5 (br), (3 × H_2O*), 6.7 (d) 6.28 (d) (p-HO—C_6H_4—), 7.37 (d) 8.1—9.3 (m, broad) (heteroaromatic protons + NH_3^+** +2 × CONH*), *exchangeable with D_2O, biochromatogram, Rf (B/E/W) ≙ 0.36, hydroxylamine assay 96.6% (versus Pen.G).

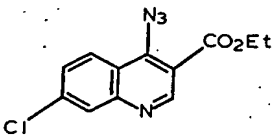
Example 7.

a) *Ethyl - 4 - azido - 7 - chloroquinoline - 3 - carboxylate.*



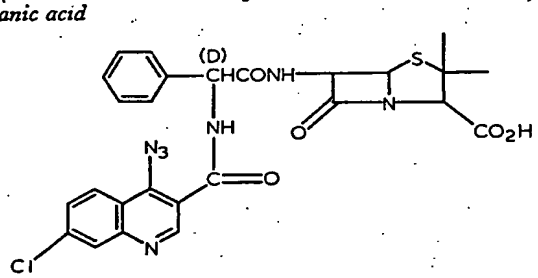
Sodium azide (0.62g; 0.009M) was suspended in a solution of ethyl - 4,7-dichloroquinoline - 3 - carboxylate E. F. Elslager *et al*, J. Med. Pharma. Chem. 5. 550 (1962) (1.75g; 0.006M) in dry dimethyl formamide (20ml) and this mixture stirred at ambient temperatures. After 18hr. the mixture was poured into rapidly stirred water (200ml), the resultant precipitate filtered off, dried at the pump and then recrystallised from ethanol (8ml/g), 1.27g (71%), m.p. 94.5° C, (Found: C, 51.7; H, 3.4; N, 20.2; Cl, 12.9% $C_{12}H_9N_4O_2Cl$ requires C, 52.0; H, 3.3; N, 20.2; Cl, 12.8%); max (KBr) 2140, 1722, 1390, 1372, 1274, 1239, 1199 and 1058cm^{-1}, ($CDCl_3$) 1.48(t) and 4.55 (m) (CH_3CH_2), 7.59 (m), 8.11 (d), 8.32 (d) and 9.29 (s) (aromatic H's) m/e 276 (M^+, 14%), 248 (9%), 218 (22%), 154 (29% 152 (100%).

b) 4 - Azido - 7 - chloroquinoline - 3 - carboxylic acid



Ethyl - 4 - azido - 7 - chloroquinoline - 3 - carboxylate (0.57g; 0.002M) in 10% aq. NaOH (10ml) and stirred at 40°C for 5hr. The unreacted ester, m.p. 91—4°C, was filtered off, the filtrate acidified to pH 3 with 5*M* HCl, the product filtered off, washed well with water and dried *in vacuo* over P_2O_5. 0.33g (66%), m.p. 284—6°C (dec.), (Found: C, 46.7; H, 2.3; Cl 14.1%. $C_{10}H_5ClN_4O_2 . \frac{1}{2}H_2O$ requires C, 46.6; H, 2.4; Cl 13.8%), υ_{max} (KBr) 2158, 1705 (br), 1608, 1562, 1395 (br), 1210 and 798cm^{-1}, δ [$(CD_3)_2SO$] 7.72 (m), 8.09 (d), 8.36 (d) and 9.18 (s) (aromatic H's).

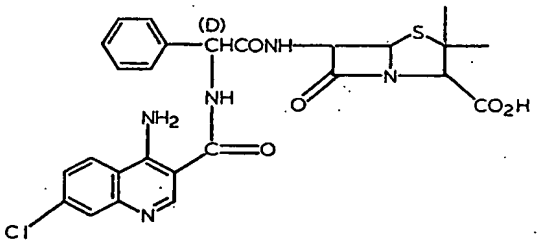
c) *6 - [D - α - (4 - Azido - 7 - chloroquinoline - 3 - carboxamido)phenylacetamido] penicillanic acid*



4 - Azido - 7 - chloroquinoline - 3 - carboxylic acid (0.7g; 0.0028M) was dissolved at 0—5° C in dry D.M.F. (25ml) and to the stirred solution was added N-hydroxysuccinimide (0.33g; 0.0028M) and N,N'-dicyclohexycarbodiimide (0.63g; 0.03M). After stirring for 1hr. at 0—5°C the reaction was allowed to regain ambient temperatures and stirred at ambient temperatures overnight. Sodium ampicillin (1.0g; 0.0028M) was added to the reaction at 0—5°C and the reaction mixture stirred at 0—5°C for 1hr. and allowed to reach ambient temperatures over ½hr. The reaction mixture was then poured into rapidly-stirred, dry Et_2O (21) and the precipitate removed by filtration, washed with dry Et_2O and immediately redissolved in H_2O (50ml). The aqueous mixture was filtered and the pH of the filtrate adjusted to 2.5 with 5M HCl in the presence of EtOAc (50ml). The layers were separated,

the aqueous phase extracted with EtOAc (2 × 50ml), the extracts combined, washed with H_2O at pH 2 (2 × 25ml), saturated brine (25ml) and dried over anhydrous $MgSO_4$. The solvent was concentrated *in vacuo*, diluted with dry Et_2O and the product collected by filtration, washed with dry Et_2O and dried over P_2O_5 *in vacuo*, 0.22g (14%), ν_{max} (KBr) 3700—3100 (br), 2238, 1775, 1730, 1650, 1608, 1520, 1379, 1300, 1212, 702cm^{-1}, δ [$(CD_3)_2SO$] 1.4 (s), 1.53 (s) (gem dimethyls), 4.19 (s) (C_3 proton), 5.3—5.6 (m) (β-lactams), 5.99 (d) (α-proton), 7.1—7.8 (m), 8.02 (d), 8.16 (d), 8.81 (s) (aromatic + heteroaromatic protons), 9.12 (d), 9.60 (d) (2 × CONH*), CO_2H* diffuse, low-field resonance, biochromatogram, Rf (B/E/W) ≏ 0.74, hydroxylamine assay 82.1% (versus Pen G.).

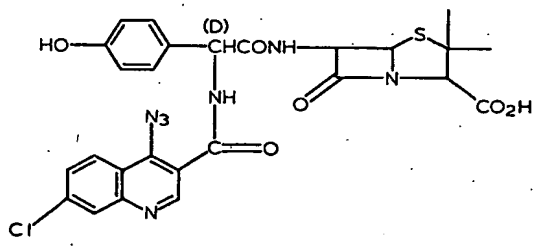
(d) *6 - [D - α - (4 - Amino - 7 - chloroquinoline - 3 - carboxamido)phenylacetamido]penicillanic acid*



6 - [D - α - (4 - Azido - 7 - chloroquinoline - 3 - carboxamido)phenylacetamido] penicillanic acid (0.64g; 0.0011M) was dissolved in H_2O (20ml) containing $NaHCO_3$ (0.09g; 0.0011M). This solution was added to a suspension in H_2O (10 ml) of 5% $Pd/CaCO_3$ (0.64g), which had been pre-hydrogenated for 1hr. at ambient temperatures and atmospheric pressure. The mixture was hydrogenated for 1¼ hr. at ambient temperatures and atmospheric pressure before the catalyst was removed by filtration through Kieselgühr and the filtrate acidified to pH 3 with 5M HCl. The product (0.2g; 30%) was collected by filtration, washed with H_2O and dried over P_2O_5 *in vacuo*, ν_{max} (KBr) 3700—2250 (br), 1765, 1700—1570, 1550, 1515, 1371, 1325, 1250, 1212, 790, 701cm^{-1}, δ[$(CD_3)_2SO$] 1.42 (s), 1.51 (s) (gem dimethyls), 4.19 (s) (C_3 proton), 5.2—6.2 (broad m) (3×H_2O*+β-lactams+α-proton), 7.0—7.7 (broad m), 7.8 (broad), 8.2—8.5 (broad), 7.6—9.3 (broad) (aromatic+heteroaromatics protons+NH_3^{+}*+2×CONH*), *exchangeable with D_2O; biochromatogram, Rf (B/E/W)≏0.74.

Example 8.

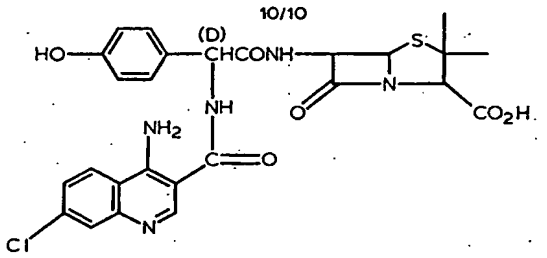
a) *6 - [D - α - (4 - Azido - 7 - chloroquinoline - 3 - carboxamido) - α - (4 - hydroxyphenyl)acetamido]penicillanic acid*



4 - Azido - 7 - chloroquinoline - 3 - carboxylic acid (0.57g; 0.0023M) was dissolved at 0—5°C in dry D.M.F. (15ml). To this stirred, cold solution was added N-hydroxysuccinimide (0.26g; 0.0023M) and N,N'-dicyclohexylcarbodiimide (0.52g; 0.0025M) and the mixture stirred at 0—5°C for 1hr. and allowed to regain ambient temperatures. The reaction was stirred at ambient temperatures overnight and then cooled to 0—5°C. Triethylammonium 6 - [D - α - amino - α - (4 - hydroxyphenyl)-acetamido]penicillanate (1.0g; 0.0021M) was added and the mixture stirred at 0—5°C for 1hr. and allowed to regain ambient temperatures over ½ hr. The reaction mixture was filtered into rapidly-stirred, dry Et_2O (2l) and the precipitate removed

by filtration, washed with dry Et_2O and immediately added to H_2O (50ml). The mixture was filtered and the pH of the filtrate adjusted to 2.8 with 5M HCl. The product (0.52g; 38%) was collected by filtration, washed with H_2O and dried over P_2O_5 *in vacuo*, ν_{max} (KBr) 3700—3100 (br), 2140, 1770, 1740, 1645, 1610, 1515, 1380, 1240, 840cm^{-1}, δ[$(CD_3)_2SO$] 1.4 (s), 1.55 (s) (gem dimethyls), 4.16 (s) (C_3 proton), 5.3—5.64 (m) (β-lactams), 5.82 (d) (α-proton), 6.68 (d), 7.28 (d) (p—HO—C_6H_4—), 7.62 (dd), 9.03 (d), 8.17 (d), 8.8 (s) (heteroaromatic protons), 8.99 (d), 9.5 (d) (2 × CONH*), OH* and CO_2H* diffuse, low-field resonances, biochromatogram, Rf (B/E/W)≃0.70, hydroxylamine assay 82.1% (versus Pen G.).

b) *6 - [D - α - (4 - Amino - 7 - chloroquinoline - 3 - carboxamido) - α - (4-hydroxyphenyl)acetamido]penicillanic acid (9)*



6 - [D - α - (4 - Azido - 7 - chloroquinoline - 3 - carboxamido) - α - (4-hydroxyphenyl)acetamido]penicillanic acid (0.5g; 0.00084M) was dissolved in H_2O (20ml) containing $NaHCO_3$ (0.07g; 0.00083M). This solution was added to a suspension of 5% Pd/$CaCO_3$ (0.5g) in H_2O (10ml) which had been pre-hydrogenated for 1hr. at atmospheric pressure and ambient temperatures. The reaction mixture was hydrogenated at atmospheric pressure and ambient temperatures for 1¼ hr. before the catalyst was removed by filtration through Kieselgühr. The pH of the filtrate was adjusted to 3 and the product removed by filtration, 0.32g (61%), washed with H_2O and dried over P_2O_5 *in vacuo*, ν_{max} (KBr) 3700—2250 (br), 1760, 1700—1560 (2 broad peaks), 1510, 1470, 1370, 1320, 1250, 1180, 913, 890, 790cm^{-1}, δ[$(CD_3)_2SO$] 1.4 (s), 1.5 (s) (gem dimethyl), 4.16 (s) (C_3 proton), 5.0—6.5 (broad) (3 × H_2O*), 5.35—5.60 (m) (β-lactams), 5.7 (d) (α-proton), 6.68 (d), 7.27 (d) (pHO—C_6H_4—), 7.34 (dd), 7.8 (d), 8.2—8.58 (br), 8.62—9.1 (br) (heteroatomic protons + 2 × CONH* + NH_3^+*), *exchangeable with D_2O, biochromatogram, Rf (B/E/W)≃0.63, hydroxylamine assay 96.2% (versus Pen.G.).

Biological Data

Table 1 and 2 show the antibacterial activity of the compounds of Examples 1—8, in terms of their minimum inhibitory concentrations (in mg/ml) against a range of organisms determined in nutrient agor. The figures in brackets represent values determined in broth.

Table 3 shows the activity of some of the compounds of the invention against a number of strains of Pseudomonas aeruginosa. For comparison purposes, the activity of ticarcillin in the same test is shown.

TABLE 1

In Vitro Primary Antibacterial Evaluation

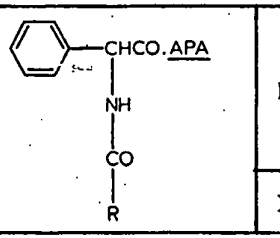
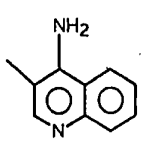
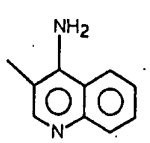
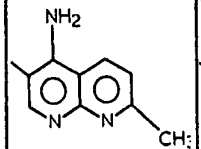
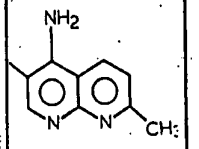
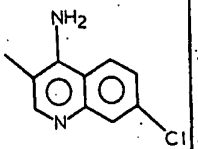
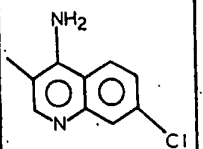
Reference No:		AB 20176	AB 20196	AB 20214	AB 20215	AB 20221	AB 20213
	R						
	X	H	HO	H	HO	H	H
Compound of Example No:		1	4	5	6	7	8
Purity (%)		~ 90%	~95%	~80%	~90%	~70%	~90%
		Minimum Inhibitory Concentrations (μg/ml)					
E. coli JT 1		5.0 (2.5)	25 (2.5)	12.5 (12.5)	5.0 (2.5)	5.0	12.5 (12.5)
E. coli JT 4		> 100	> 500	> 500	> 500	> 500	> 500
E. coli JT 425		25	25	50	50	12.5	25
E. coli NCTC 10418		2.5 (1.0)	25 (2.5)	5.0 (2.5)	5.0 (2.5)	12.5	12.5 (2.5)
Ps. aeruginosa 10662 nt.		10 (10)	25 (2.5)	12.5 (12.5)	5.0	50	125 (12.5)
Ps. aeruginosa 10662 10^{-2}		2.5 (2.5)	12.5 (2.5)	0.5 (5.0)	2.5	5.0	5.0 (1.2)
Ps. aeruginosa Dalgleish 10^{-2}		25	25	50	50	25	25
Serratia narcescens US 32		10	500	25	50	12.5	25
Klebsiella aerogenes A		50	500	50	125	50	125
Enterobacter cloacae N1		10	25	12.5	12.5	12.5	12.5
P. mirabilis C977		5.0	50	5.0	5.0	5.0	5.0
P. mirabilis 899		> 100	> 500	> 500	> 500	> 500	> 500
P. morganii		10	25	12.5	12.5	12.5	12.5
P. rettgeri		25	25	12.5	12.5	25	25
B. subtilis		2.5	12.5	0.5	1.2	1.2	1.2
Staph. aureus (Oxford)		0.2 (1.0)	1.2 (0.2)	2.5 (0.5)	2.5 (0.5)	1.2	1.2 (0.5)
Staph. aureus (Russell)		> 100	> 500	> 500	500 (250)	500	250
Staph. aureus 1517		> 100	–	> 500	500	500	500
Strep. faecalis I		1.0	5.0	1.2	1.2	1.2	1.2
β-Haemolytic Strep CN10		≤0.02	0.5	≤0.02	0.05	≤0.02	≤0.02

TABLE 2

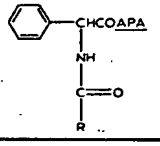
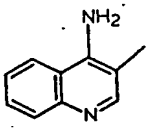
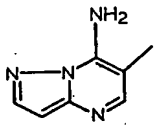
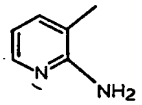
Reference No:		20176	20115	20063
	R			
Compound of Example number:		1	2	3
Purity (%) :		70	75	90
E. coli JT 1		5.0(2.5)	25(25)	125(50)
E. coli JT 4		>500	>250	>500
E. coli JT 425		25	125	500
E. coli NCTC 10418		2.5(1.2)	25(12.5)	125(12.5)
Ps. aeruginosa 10662 nt.		–	50(125)	50(125)
Ps. aeruginosa 10662 10^{-2}		2.5(2.5)	12.5(25)	50(50)
Ps. aeruginosa Dalgleish 10^{-2}		50	125	250
Serratia marcescens US 32		25	–	125
Klebsiella aerogenes A		125	125	125
Enterobacter cloacae N1		5.0	12.5	125
P. mirabilis C977		2.5	12.5	50
P. mirabilis 889		>500	>250	>500
P. morganii		12.5	250	>500
P. rettgeri		25	50	500
B. subtilis		5.0	5.0	2.5
Staph. aureus Oxford		<0.1(0.01)	5.0	0.2(1.2)
Staph. aureus Russell		250	>250	250(>500)
Staph. aureus 1517		250	>250	>500
Strep. faecalis I		0.5	5.0	1.2
β-Haemolytic Strep. CN10		<0.1	0.5	0.01

TABLE 3

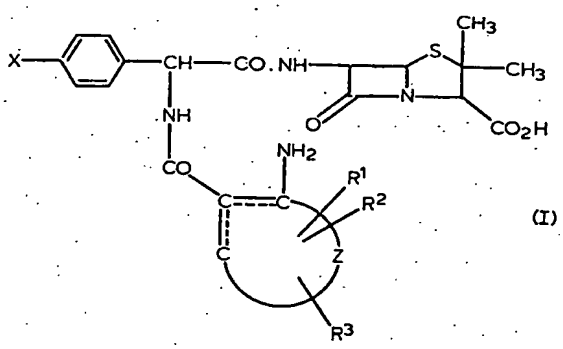
MIC* (mg/ml) and No. of Strains

Example No.	Inoculum	1.2	2.5	5.0	12.5	25	50	125	250	500
8		5		3	2	2	2	1	2	3
5		1	6	5	3	2	2	1		
	Undiluted									
6		1	4	9	5			1		
4			2	1	1	1	2	2	2	9
Ticarcillin				4	4	3	6		2	1
8		11	4	3	2					
5		8	10	1	1					
	Diluted 1/100									
6		5	9	4	2					
4		5	12	1	2					
Ticarcillin				6	9	3				

* Serial dilution in nutrient agar. inoculum 0.001 ml. o.b.c. diluted as specified.

WHAT WE CLAIM IS:—

1. A penicillin of formula (I) or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof:



(I)

wherein X is hydrogen or hydroxy;

the dotted line represents a double bond in one of the positions shown;

Z represents the residue of a 6-membered heterocyclic ring containing one or two nitrogen atoms;

R^1, R^2 and R^3 are the same or different and each represents hydrogen, halogen, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkylthio, cyano, amino, mercapto, C_{1-6} alkylamino, di-C_{1-6} alkylamino, C_{1-6} alkanoyl-amino, nitroformyl or hydroxy or any two of R^1, R^2 and R^3 on adjacent carbon or nitrogen atoms represent the residue of a fused 5- or 6-membered carbocyclic or heterocyclic ring containing up to three heteroatoms selected from oxygen, sulphur and nitrogen, and being optionally substituted with up to three substituents selected from halogen, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkylthio or hydroxy, and the remaining symbol is as defined above

2. A penicillin as claimed in claim 1 wherein Z represents the residue of a pyridine or pyrimidine ring.

3. A penicillin as claimed in either claim 1 or 2 wherein R^3 is hydrogen.

4. A penicillin as claimed in any one of claims 1 to 3 wherein R^1 and R^2 together represent the residue of a fused 5- or 6-membered carbocyclic or nitrogen-containing heterocyclic ring.

5. A penicillin as claimed in claim 4 wherein the residue formed by R^1 and R^2 is optionally substituted with a halogen or C_{1-6} alkyl group.

6. 6 - [D - α - (4 - Aminoquinolin - 3 - carboxamido)phenylacetamido]penicillanic acid.

7. 6 - [D - α - (4 - Aminoquinolin - 3 - carboxamido) - 4 - hydroxyphenylacetamido] penicillanic acid.

8. 6 - [D - α - (7 - Aminopyrazolo[1,5 - a]pyrimidine - 6 - carboxamido]-phenylacetamido penicillanic acid.

9. 6 - [D - α - Aminopyrazolo[1,5 - a]pyrimidine - 6 - carboxamido] - 4-hydroxyphenylacetamido penicillanic acid.

10. 6 - [D - α - (2 - Aminopyridine - 3 - carboxamido)phenylacetamido]penicillanic acid.

11. 6 - [D - α - (2 - Aminopyridine - 3 - carboxamido)4 - hydroxyphenylacetamido]penicillanic acid.

12. 6 - [D - α - (4 - Amino - 1,5 - naphthridine - 3 - carboxamido)phenylacetamido]penicillanic acid.

13. 6 - [D - α - (4 - Amino - 1,5 - naphthridine - 3 - carboxamido) - 4 - hydroxyphenylacetamido]penicillanic acid.

14. 6 - [D - α - (3 - Aminopyridazine - 4 - carboxamido)phenylacetamido]-penicillanic acid.

15. 6 - [D - α - (3 - Aminopyridazine - 4 - carboxamido) - 4 - hydroxyphenylacetamido]penicillanic acid.

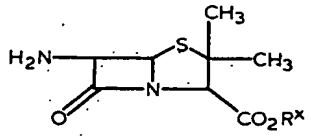
16. 6 - [D - α - (4 - Amino - 7 - methyl - 1,8 - naphthridine - 3 - carboxamido)phenylacetamido]penicillanic acid.

17. 6 - [D - α - (4 - Amino - 7 - methyl - 1,8 - naphthridine - 3 - carboxamido)-4 - hydroxyphenylacetamido]penicillanic acid.

18. 6 - [D - α - (4 - Amino - 7 - chloroquinoline - 3 - carboxamido)phenylacetamido]penicillanic acid.

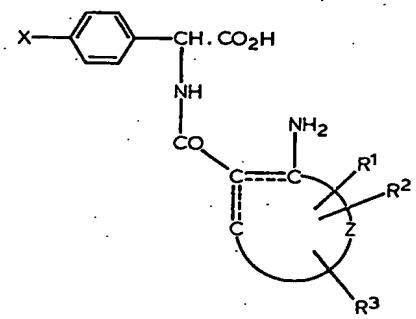
19. 6 - [D - α - (4 - Amino - 7 - chloroquinoline - 3 - carboxamido) - 4-hydroxyphenylacetamido]penicillanic acid.

20. A process for the preparation of a penicillin as claimed in claim 1 which process comprises (a) reacting a compound of formula (III) or an N-protected derivative thereof which allows acylation to take place:



(III)

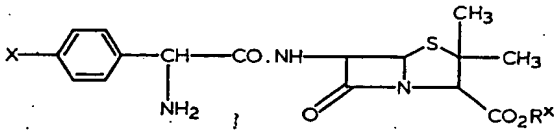
wherein R^x is hydrogen, an *in vivo* hydrolysable ester radical or a carboxyl blocking group; with an N-acylating derivative of an acid of formula (IV):



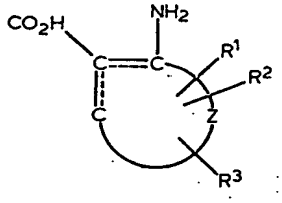
(IV)

wherein X, Z, R^1, R^2 and R^3 as defined in claim 1, and wherein any amino and hydroxy group may be blocked;

or (b) reacting a compound of formula (XI) or an N-protected derivative thereof which allows acylation to take place:



wherein X is as defined in claim 1 and R^x is a carbonyl blocking group; with an N-acylating derivative of an acid of formula (IX):



(IX)

wherein Z, R^1, R^2 and R^3 are as defined in claim 1 and wherein the amino and any hydroxy groups may be blocked; and after step (a) or step (b), if necessary carrying out one or more of the following steps:

(i) removal of any N-protecting groups by hydrolysis or alcoholysis;
(ii) removal of any carboxyl blocking groups;
(iii) removal of any amino or hydroxy blocking groups;
(iv) converting the product to a salt or ester thereof.

21. A process as claimed in claim 21 substantially as described in any one of Examples 1 to 8.

22. A penicillin as claimed in claim 1 whenever prepared by a process as claimed in either claim 21 or 22.

23. A pharmaceutical composition comprising a pharmaceutically acceptable carrier together with at least one penicillin as claimed in claim 1.

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Chartered Patent Agent.

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